ted. This method affords a convenient way to evaluate the relative activity of various adrenal cortical steroids and adrenotrophic hormone. Further studies are needed to define the method as a quantitative assay for adrenal cortical steroids.

Summary. By the use of radiopotassium in the adrenalectomized male rat, it is possible to detect as little as 10 μg of desoxycorticosterone. The method affords a convenient means of evaluating the relative activities of adrenal cortical steroids, as well as the influence of adrenotrophic hormone on potassium metabolism.

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Time of Appearance of Antibodies to Brain in the Human Receiving Anti-Rabies Vaccine.

R. C. KIRK AND E. E. ECKER.

From the Institute of Pathology and the Department of Ophthalmology of Western Reserve University and University Hospitals of Cleveland, Ohio.

Recent reports[1-12] have shown that a disseminated type of encephalomyelitis can be produced in experimental animals by the injection of homologous and heterologous brain tissue, and that the lesions produced resemble the lesions found in the human demyelinating diseases including the encephalomyelitis following anti-rabies vaccination.

This has suggested the possibility that isoimmunization to brain tissue may be the mechanism responsible for the production of these diseases, and, in particular, of the encephalomyelitis following anti-rabies vaccination, since in the latter, the injection of heterologous brain tissue duplicates in many ways the actual experimental procedure which has been followed with the experimental animals.

It seemed of interest, therefore, to determine, in a preliminary study (of 5 cases) whether or not patients receiving anti-rabies vaccine actually develop antibrain antibodies in their sera. The procedure followed consisted of (1) the immunization of rabbits to determine the antigenicity of the brain extract used, and (2) the titration of the sera of patients receiving the anti-rabies vaccine, with this particular antigen.

Materials and methods. I. Preparation of antigens (Lewis[13]):

A. Suspension. The white matter from fresh human brain was dissected free of cortex, connective tissue and blood vessels, cut into thin slices and washed in running water for 30 minutes. It was then ground in a Waring blendor and made into a 30% saline suspension with 0.5% phenol added. This stock solution was used in the immunization of 4 rabbits.

B. Alcoholic extract. The same procedure

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TABLE I.
Complement Fixation Titers Obtained in Rabbits Given Intravenous Injections of Human Brain Mixed with Normal Rabbit Serum.

Rabbit No.	Highest titers obtained			
	8 days	16 days	24 days (Inj. dis.)	40 days
889	1/20	1/40	1/45	1/10
979	1/10	1/30	1/40	1/10
991	1/5	1/20	1/40	1/20
987	1/10	1/30	1/30	1/20

was followed except that after grinding the brain tissue in the Waring blendor, the material was mixed with 10 times its volume of 95% ethyl alcohol and kept in the incubator at 37°C for at least 2 weeks.

II. Immunization of animals (Lewis). The rabbits were given intravenous injections of 1.5 ml of the 1:30 brain suspension every 4 days for 24 days. The suspension was mixed with an equal volume of fresh normal rabbit serum, incubated 2-3 hours at 37°C, and the mixture injected very slowly. The rabbits were bled once every 8 days from the marginal ear vein, the blood allowed to clot, the serum separated by centrifugation and frozen at –30°C until used.

III. Technic of complement fixation. The alcoholic extract alone was used as the antigen, both in the case of the rabbit sera, and in the subsequent fixation with human sera. It was found that a 1:40 dilution of the alcoholic extract in 0.9% saline served adequately without being hemolytic or anticomplementary, and without showing non-specific complement fixation with normal human sera. The amount of antigen per dry weight and in aliquot samples of the extract was checked periodically, and was found to remain relatively constant at 3 mg of solids per ml of the extract. In all instances, 0.1 ml of the 1:40 dilution of the alcoholic extract was used as the antigen; 0.1 ml of the inactivated serum, as well as of each dilution, and two units of guinea pig complement were added and the tubes kept in the refrigerator at 3-4°C overnight. The tubes were then allowed to stand at room temperature for one hour; 1.0 ml of a 2.5% suspension of sensitized sheep red cells added, and the tubes incubated at 37°C for 30 minutes. The results were recorded as 0, 1, 2, 3, and 4 plus fixation. The usual controls (antigen, antiserum, normal serum) were set up at each titration. In addition, the antigen was periodically checked against both human and rabbit positive sera.

IV. Collection of sera from patients. 10-15 ml of blood was withdrawn from patients receiving the Semple anti-rabies vaccine at the beginning of treatment, and, as nearly as possible, every two to three days thereafter. The blood was allowed to clot, the serum separated by centrifugation, and frozen at –30°C until used.

Experimental. I. Four rabbits, each weighing about 5 lbs, were given 6 intravenous injections of 3 ml of the human brain suspension mixed with normal rabbit serum at 4-day intervals as described. Control sera obtained at the start of the injections showed a + fixation of complement when 0.1 ml of the undiluted serum was used, but no fixation was observed in a 1:5 or higher dilution. The subsequent titers obtained are shown in the accompanying table.[1] It is to be noted that the injections were discontinued on the 24th day. The sera obtained from these animals were used as controls in the subsequent fixation tests with human sera.

II. The sera from the 5 patients receiving the anti-rabies vaccine were then titrated in a similar manner. The results obtained are shown in Fig. 1 and 2.

Discussion of cases. Cases No. 1, 3, and 4. These patients were given the routine 14 daily injections of the Semple anti-rabies vaccine (25% suspension phenol-killed rabbit brain virus) following a dog bite in which the dog was not found for examination. They tolerated the injections well and showed very

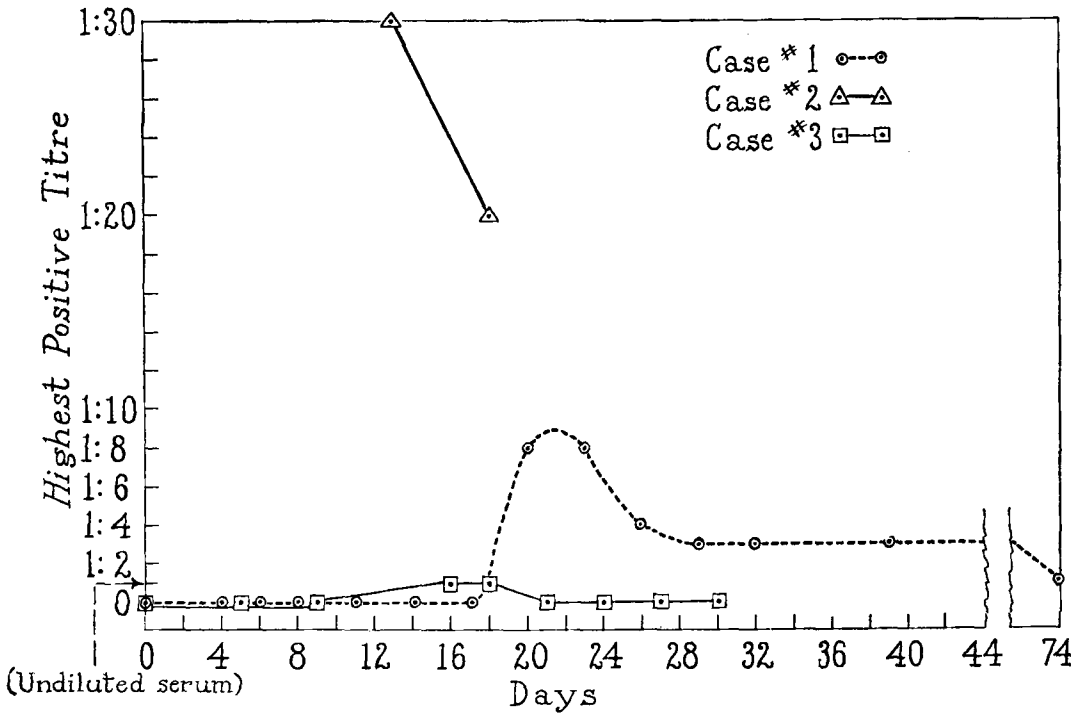

FIG. 1.
Complement fixation titers in cases 1, 2, and 3.

little reaction. Cases 3 and 4 developed some swelling and redness at the site of the injections between the 10th and 14th dose. In each case, the sera gave a negative complement fixation reaction until the 15th to 18th day at which time antibodies to the brain antigen appeared. (See Fig. 1 and 2).

Case No. 2. This patient was a 9-year-old Italian boy who was bitten by a dog on August 17, 1948. The dog was not found and for this reason injections of the antirabies vaccine were started on August 23, 1948. He received a total of 5 injections, each of which produced considerable swelling and redness at the site of injection. Because of the discomfort, the patient and his parents objected strenuously to the treatment, and the patient was discharged against medical advice. He had given no previous history of allergy, and his initial physical examination was entirely normal. On August 29, 1948, 6 days after the first injection with the antirabies vaccine, the patient became lethargic, vomited, developed a fever, anorexia, and pain in the right upper abdominal quadrant. He was readmitted to the hospital where his temperature was found to be 39.2°C, pulse 120, and his appearance lethargic. He appeared "quite ill." Examination revealed a mild inflammation of the nasopharynx, a stiff neck, and a positive Kernig and Brudzinski. The total white count on admission was 9,800 with a slight leukocytosis. The spinal fluid showed 23 cells, of which 18 were described as monocytes and 5 as polymorphonuclear leukocytes. The CSF protein was 70, sugar 100, and chlorides (as NaCl) 734.

It was possible to obtain only 2 samples of his blood for titration, one on September 7th, 9 days following the development of symptoms, and the other 3 days later. Both samples showed a high titer of complement fixation when compared to the other cases studied, the second blood sample showed a falling titer. Apparently, the antibody titer had begun considerably earlier than in the other four cases, and it may well have reached an even higher concentration than that found in the sample of September 7th. The patient's symptoms subsided and he was dis-

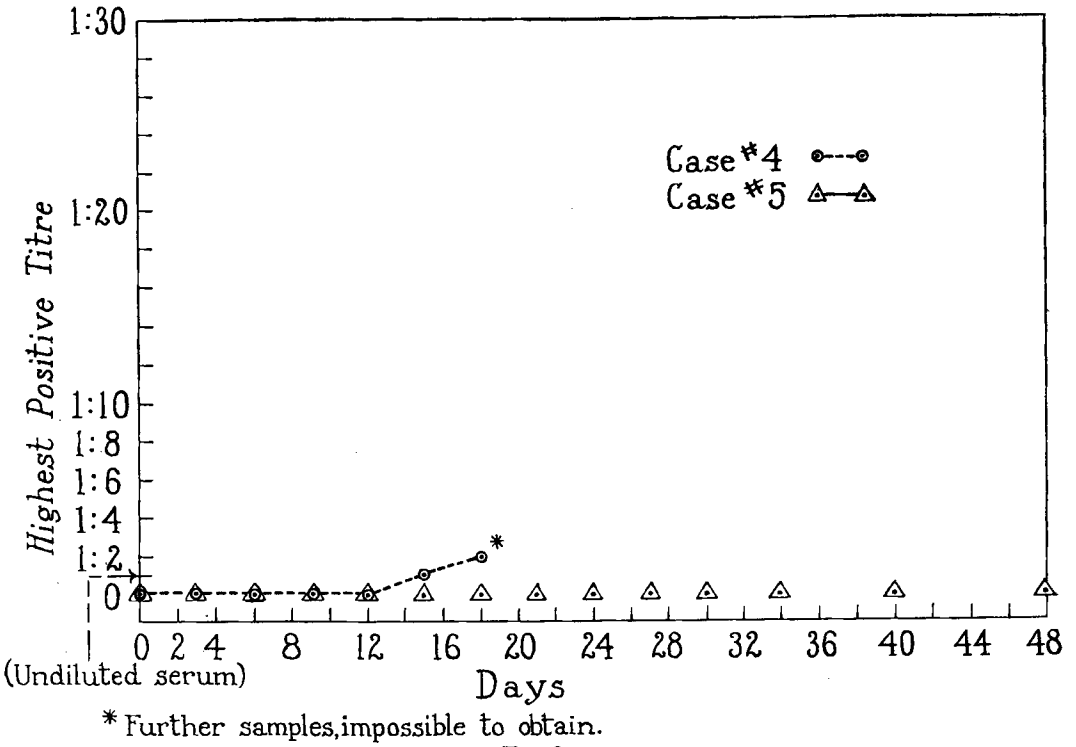

FIG. 2.
Complement fixation titers in cases 4 and 5.

charged entirely well on his 14th hospital day, with the provisional diagnosis of "possible encephalitis following anti-rabies treatment."

Case No. 5. This 34-year-old white female was bitten on the face by a stray dog. She was given the routine 14 daily injections of the vaccine and showed only a slight amount of redness and swelling at the site of injections between the 10th and 14th day of treatment. Blood samples obtained up to the 60th day after the beginning of treatment failed to show fixation of complement. The tests on the sera of this patient were repeated with varying dilutions of the antigen from 1 to 20 up to 1 to 1280, but no fixation occurred. It was then thought that the patient's sera might contain an inhibiting substance. In order to determine this, portions of these same sera (0.3 ml) were mixed with equal portions of the fresh positive serum of Case No. 2. This mixture was inactivated at 55°C for 30 minutes and varying dilutions were then used in fixation reactions with the brain antigen as previously described. Controls of the known positive serum diluted in the same manner with a normal serum, and of the positive serum alone were run at the same time. No inhibition of complement fixation was found to occur with the sera of case No. 5 or with the controls. It was therefore thought that patient No. 5 failed to show antibrain antibodies following treatment with antirabies vaccine.

Comment. The present work confirms that of Lewis in showing that antibodies to brain tissue are produced in the rabbit by the intravenous injection of foreign brain substance. It also provides evidence that antibodies to human brain are produced in patients receiving the Semple antirabies vaccine. Case 2 suggests the possibility that a markedly increased antibrain antibody titer may occur in those patients developing an encephalitis following this treatment.